

FIG. 1

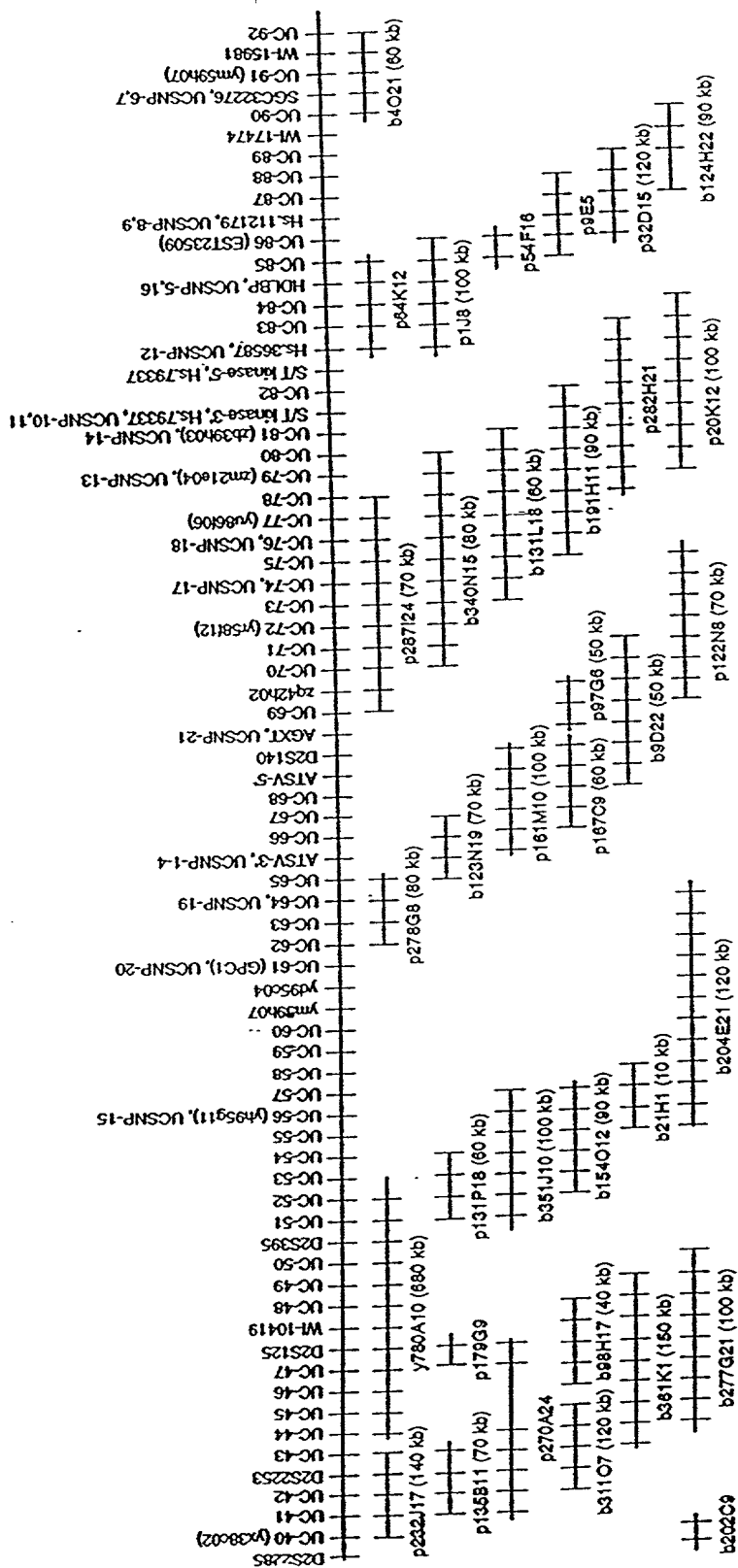


FIG. 2

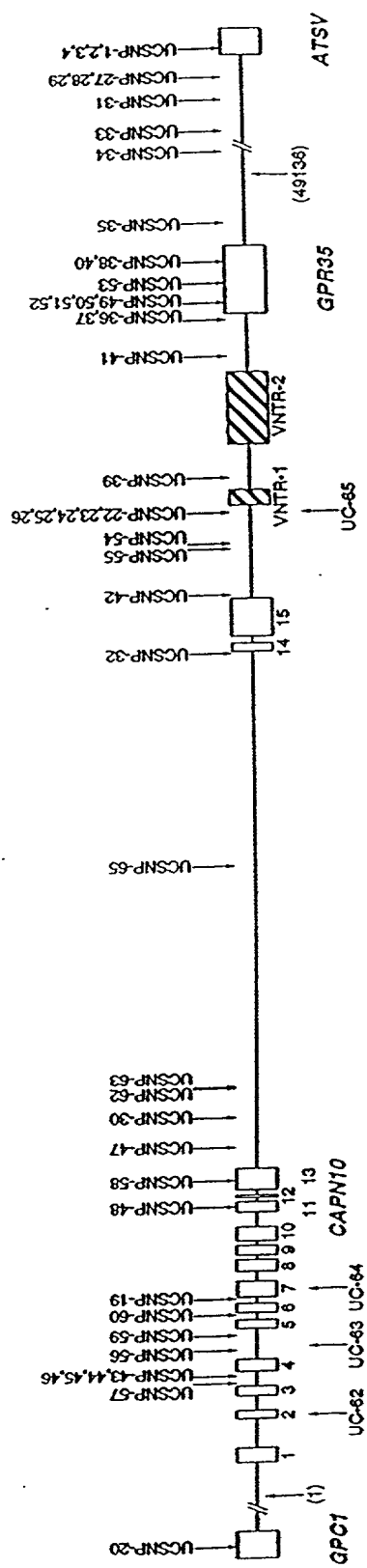


FIG. 3

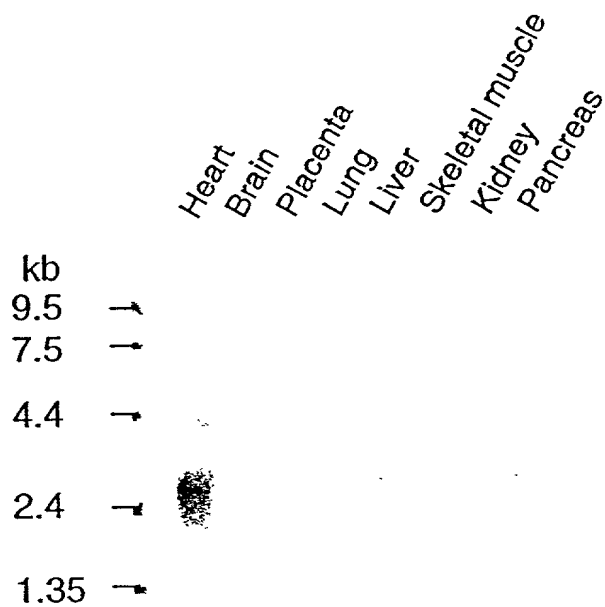


FIG. 4

		Domain I	Domain II
hCAPN5	-----HFCSCVPTEDQNYSAIRGQDQRRKRVLFEDPLFPAIDDSLYY-KGTGPE-----AVRMGRPGKICEDPRFLVDG	67	
mCAPN6	-----HGPPLKLFKNQYQELAQDCKHGRLECDFTFLPNDSLFNRLLPG-----KVMWRPQDISDOPHILVGN	67	
hCAPN3	MPVTSASVAPRTAAEPRSPCPVPHPAQSKATEAGGNGPSGIYSALISRNFLIIGVKEKTFDQAKKCLEKVLVDPEFPDQTSIFYSQKFF-----IQFVWRPPEICEINERFIIDG	115	
hCAPN9	MPVLY-----RAPCPQAPVP-----KDARITHSSGQSFQMRQECLEQRTLEDADEFPASNSSLFYSERFQ-----IPFVWRPPEIVKNEPFILOG	83	
hCAPN1	-----HSEELITFVYTCVSAQVQKQARELGLG-----RHENAKYLGQDYQELARVCLQSGTLFRDEAFPPVPSLGYQDLGPNSSKTYGLMRKRETELLSNPQFIVDG	101	
hCAPN2	-----KAGIAAKLAKDREAAGLG-----SHERAIKYLKQDYQELARVCLQSGTLFRDEAFPPVPSLGYQDLGPNSSKTYGLMRKRETELLSNPQFIVDG	91	
rCAPN8	-----HAAIAAGVSKQRAVABGLG-----SMQNAVYLGQDYQELARVCLQSGTLFRDEAFPPVPSLGYQDLGPNSSKTYGLMRKRETELLSNPQFIVDG	91	
hCAPN10	-----HRAGRGATPARELFDAFAFPADSSLFCDLSTPLAQFREDITWRPPEICATPRFLPDD	59	
hCAPN5	ISSHDLIQQQVGNMVEVAACSSLASRESLAKQVIPPWKEQEMPRKACAYAGIFHFHFRRLG-HVDVVIDERLPTVNMQLTYCHSNRNEFWALWEKAYAKLAGCCYQALDGGNTADALY	186	
mCAPN6	ISIHQLIQGRLGNKAMISAFSCLAVQESHWTKAIPNRKQDQEMPRKPEKYAGIFHFHFRHFGEMTEVVVIDOLLPITNGDLVFSFSTSHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	187	
hCAPN3	ANRDIQGGELGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	229	
hCAPN9	ATRIDIQGGELGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	197	
hCAPN1	ATRIDIQGGELGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	215	
hCAPN2	ATRIDIQGGELGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	205	
rCAPN8	ATRIDIQGGELGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	205	
hCAPN10	PRGGVYKQGLGDCHLLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQFQYKGEVVDVVIDOLLPITNNQVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	175	
hCAPN5	DFTOGVSEPIDLTGGDFANDETQRNQLFERMLKVSRLGLSASIKAV-----TAAEK-----EARLACGLVKGHAYAVTDV	258	
mCAPN6	DFTOGLAEIDHQRGRTDVAEEKYKLPGLYKTFIKGLIQCISIESP-----SQEDQ-----EVETDGLVKGHAYAVTDV	259	
hCAPN3	DFTOGVSEPIDLTGGDFANDETQRNQLFERMLKVSRLGLSASIKAV-----TAAEK-----EARLACGLVKGHAYAVTDV	341	
hCAPN9	DFTOGVSEPIDLTGGDFANDETQRNQLFERMLKVSRLGLSASIKAV-----TAAEK-----EARLACGLVKGHAYAVTDV	261	
hCAPN1	DFTOGVSEPIDLTGGDFANDETQRNQLFERMLKVSRLGLSASIKAV-----TAAEK-----EARLACGLVKGHAYAVTDV	279	
hCAPN2	DFTOGLAEIDHQRGRTDVAEEKYKLPGLYKTFIKGLIQCISIESP-----SQEDQ-----EVETDGLVKGHAYAVTDV	269	
rCAPN8	DFTOGVSEPIDLTGGDFANDETQRNQLFERMLKVSRLGLSASIKAV-----TAAEK-----EARLACGLVKGHAYAVTDV	269	
hCAPN10	DFTOGLAEIDHQRGRTDVAEEKYKLPGLYKTFIKGLIQCISIESP-----SQEDQ-----EVETDGLVKGHAYAVTDV	245	
hCAPN5	RVRLGHGLLAFFSEKLDHILRNPNWGERENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	373	
mCAPN6	RKRLGERLNVSEFTEKLYNVLRLNPLCRQENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	371	
hCAPN3	DEVFF-----KGEVKVILRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	444	
hCAPN9	DQVSE-----RGQRILRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	364	
hCAPN1	KQVNY-----RGQVSLRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	381	
hCAPN2	EEVES-----HGLQELRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	371	
rCAPN8	EEVNE-----HGRPELRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	371	
hCAPN10	RELQG-----QAGQCILLRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	352	
hCAPN5	GGCINHKDITFFNQPYIFEVKPED-----EVLCIQQPKRSTRBCKGENLAIGFDIYKVE-----E-----NRQYRHESL-----QKKAASSIYKINSRVSFLRTDQPGRYVIPT	472	
mCAPN6	GGCINNRDITFLNQPYIFEVKPED-----KVINSLQQRDLRTTRHGRDNYITIGFELKVE-----H-----NRERLRLYQERAGTSITIDTRVFLSKLKGSSYVVT	472	
hCAPN3	GGCINRDPITFQNYQLKLEEDD-----DPDSEV-ICSEFLVALHQRNRRKRLG-ASLETIGAIYVPEKHHGRK-QHLQDQFLYNASTAKSKYIDRQVSRFLPPESEVTVPS	559	
hCAPN9	DQVSE-----RGQRILRLNPNWQVENHNPWSDTSEEMQVSKSEREKHGVTVQDQGEFVTFEDVCRITFDITIKCRVINTSHL-SIHKTWEARL-----HGMATLHEDPRQNRG	471	
hCAPN1	GGCINRDPITFQNYQLKLEEDD-----DEEDGES-GCTFLVGLIQHRRRRQRHKG-EDMHTIGFGLYVPEELSGQINILHLSKNFFLNNRARSDDITINLREVLNRFLPPEVTVPS	499	
hCAPN2	GGCINRDPITFQNYQLKLEEDD-----DEEDGES-GCTFLVGLIQHRRRRQRHKG-EDMHTIGFGLYVPEELSGQINILHLSKNFFLNNRARSDDITINLREVLNRFLPPEVTVPS	487	
rCAPN8	GGCINRDPITFQNYQLKLEEDD-----DEEDGES-GCTFLVGLIQHRRRRQRHKG-EDMHTIGFGLYVPEELSGQINILHLSKNFFLNNRARSDDITINLREVLNRFLPPEVTVPS	489	
hCAPN10	GGCINRDPITFQNYQLKLEEDD-----DEEDGES-GCTFLVGLIQHRRRRQRHKG-EDMHTIGFGLYVPEELSGQINILHLSKNFFLNNRARSDDITINLREVLNRFLPPEVTVPS	471	
hCAPN5	TFEPGHGTGFLRVFTDVPNSCRELRLDEPHIT-----C-----KSSLCQYQLATQVHVLAAGLKD-----	530	
mCAPN6	MFHQGRITSEFLRLIFSEAFVQLRELTLDRPKMS-----C-----KMLARGYTKVWQITVHSADELEKYY-----	532	
hCAPN3	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	479	
hCAPN9	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	546	
hCAPN1	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	572	
hCAPN2	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	559	
rCAPN8	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	562	
hCAPN10	TFEPHQEGEFLRVFSEKRLNSEEVENTISVDPRVKKRTKPIIFVSDRANSKRELGVQDQSEEDGKGRKSPKQKQSPQPGSSDQESSEDDQGFNNIFKQIAGDQKEDALEKVLAT	541	
hCAPN5	-----SPTGANSYVILKCEGQKQVSAVQKG-----TSTPEIRNKGIFIRKLSQPIITVQVWNR-----VLKDEFLQVHLKADPDLQALATLHEDNSRQ	618	
mCAPN6	-----ANETVNPYLILKCGKEEVRSPVQRN-----TVHAIFDTQALFYRRTDIPILIIQVWNR-----KPCDQFLQVYVLDADSDCRDLKSLYLKKGKPT	620	
hCAPN3	VVNRKRLKTHGTFLESCHSHALMDTDSGKRLNQEFHILANKIKAMQKLFKHDTDQSGSTINSTEHRNAVNDAGFHLARQVYDITRATADKRRHEDFUSFOCVRLBGEHRAHAF	795	
hCAPN9	VVNRKRLKTHGTFLESCHSHALMDTDSGKRLNQEFHILANKIKAMQKLFKHDTDQSGSTINSTEHRNAVNDAGFHLARQVYDITRATADKRRHEDFUSFOCVRLBGEHRAHAF	666	
hCAPN1	VLAKRQDIKSDGFSIETCKIRVHDLSDSGSKLGLKEFYIATKIQYKQYKTYREDVDRSGTHSRHKALEEAGFHPQQLHQVTVARFADQQLITDQFNVRCILVLETFLKIFKL	692	
hCAPN2	VLAKRQDIKSDGFSIETCKIRVHDLSDSGSKLGLKEFYIATKIQYKQYKTYREDVDRSGTHSRHKALEEAGFHPQQLHQVTVARFADQQLITDQFNVRCILVLETFLKIFKL	687	
rCAPN8	VLAKRQDIKSDGFSIETCKIRVHDLSDSGSKLGLKEFYIATKIQYKQYKTYREDVDRSGTHSRHKALEEAGFHPQQLHQVTVARFADQQLITDQFNVRCILVLETFLKIFKL	687	
hCAPN10	-----PCFFSVPEGGRPCRVITLHQRKPSD-----TEFPIGRTVQVPEGSSQAPPLILLQVHRYAQVSRILCLLPAGTYKVVVSTLITDQGAFTVITATRIDRPSIHSG	65	
hCAPN5	PSNLPGTVAVHILSSSTSLNAV- 639		
mCAPN6	AKVKQGHISFVSISSDOLTEL- 641		
hCAPN3	DKDGGGILKINVLWGLQTHYA 821		
hCAPN9	STNKEFTHLNLINEFILHTRN1 690		
hCAPN1	DTLDGCVTFDLFKMLQTHYA 714		
hCAPN2	DPENTGTELDLSWLCFSVL- 708		
rCAPN8	DKDGGGIVQLSLAELWCCVLY- 703		
hCAPN10	ELDQGLQGVSRVAVKRT- 672		

Fig. 5

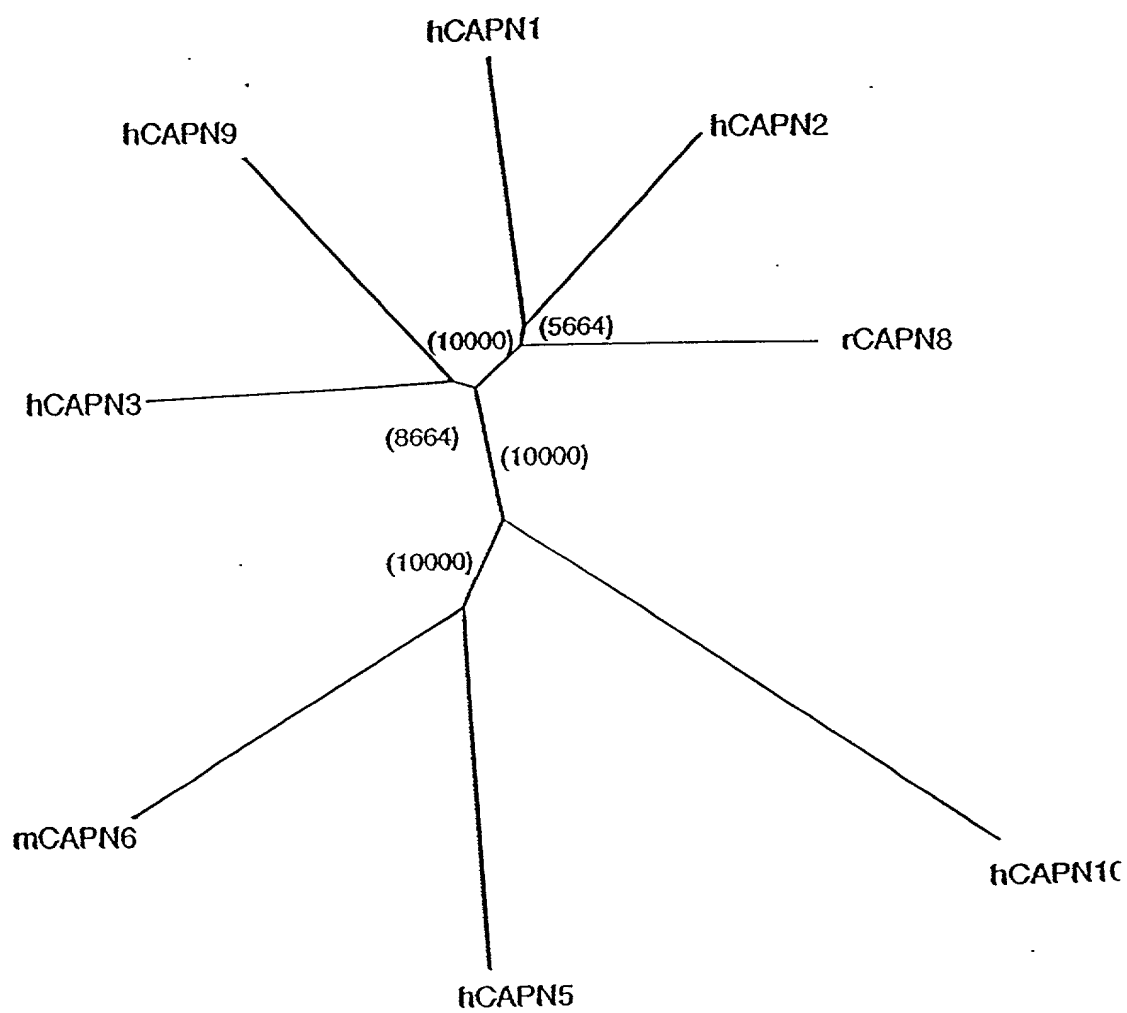


FIG. 6

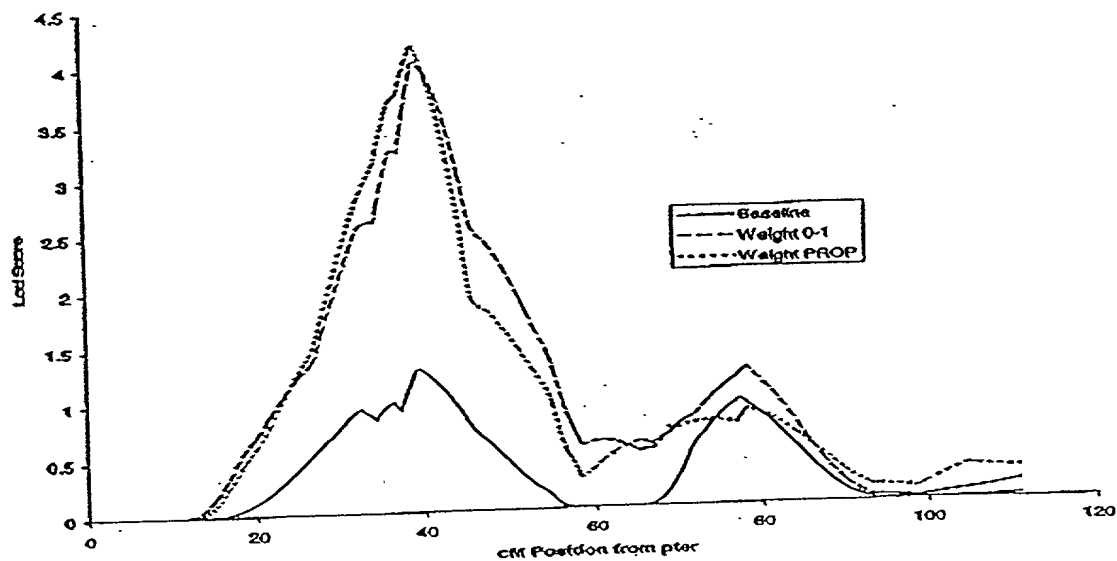


FIG. 7A

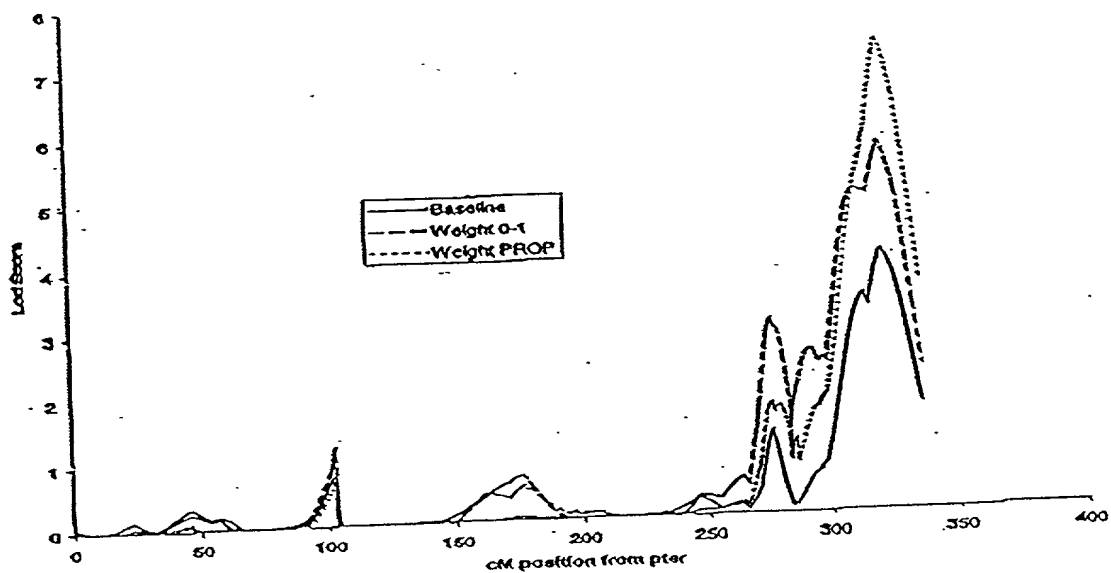


FIG. 7B

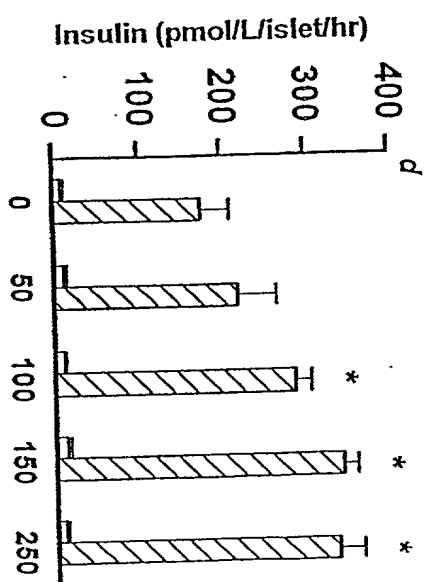
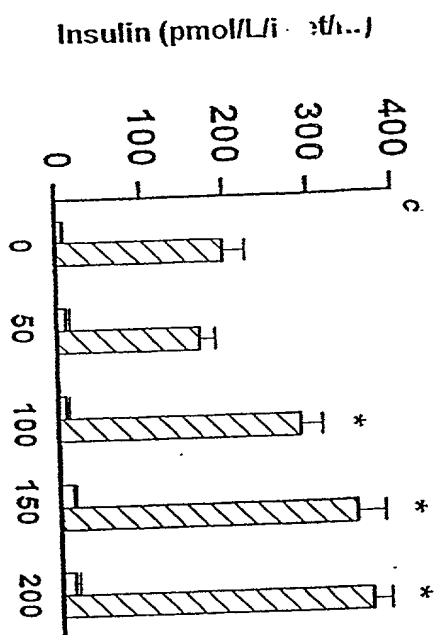
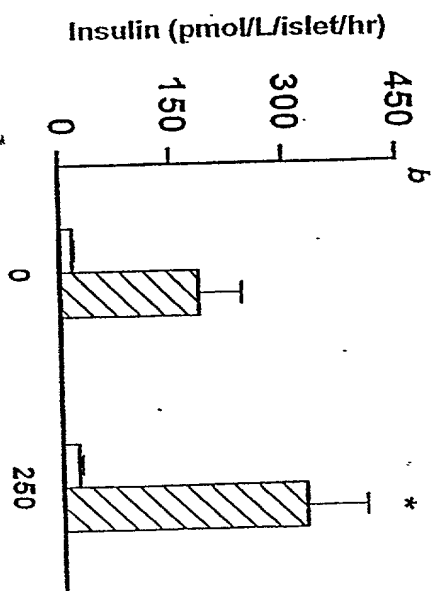
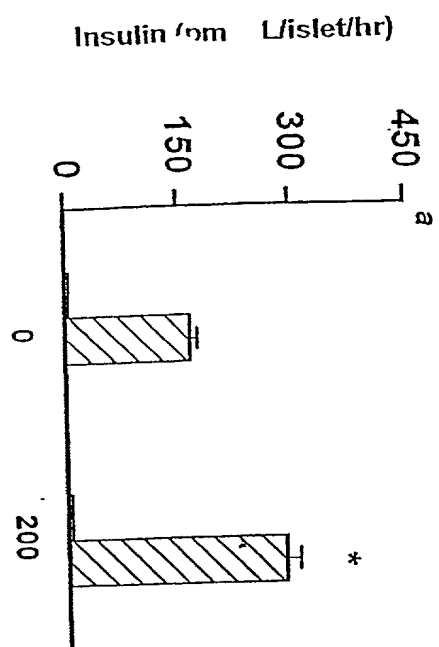


FIG. 8



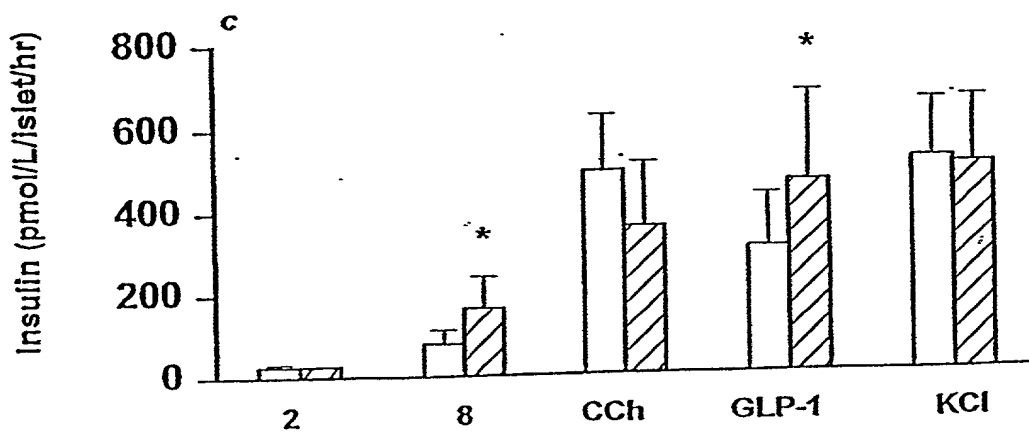
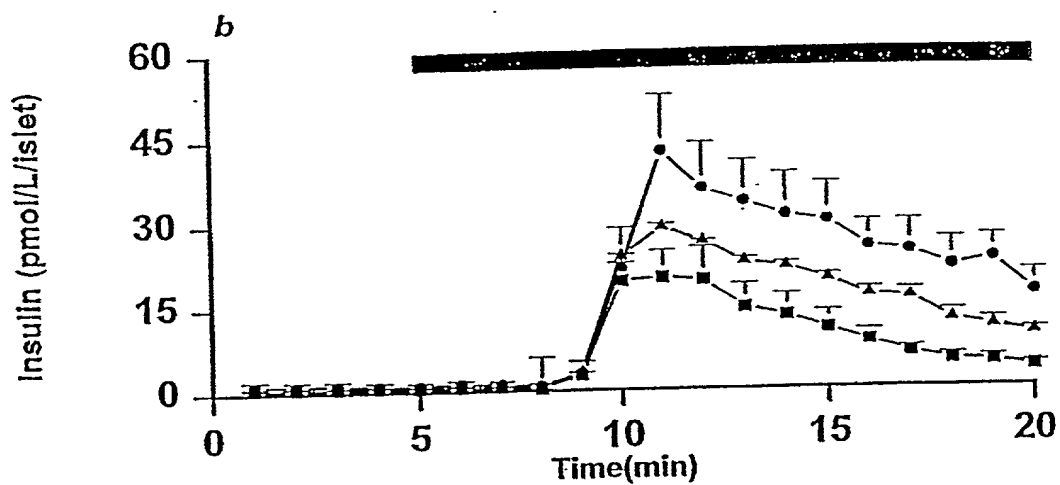
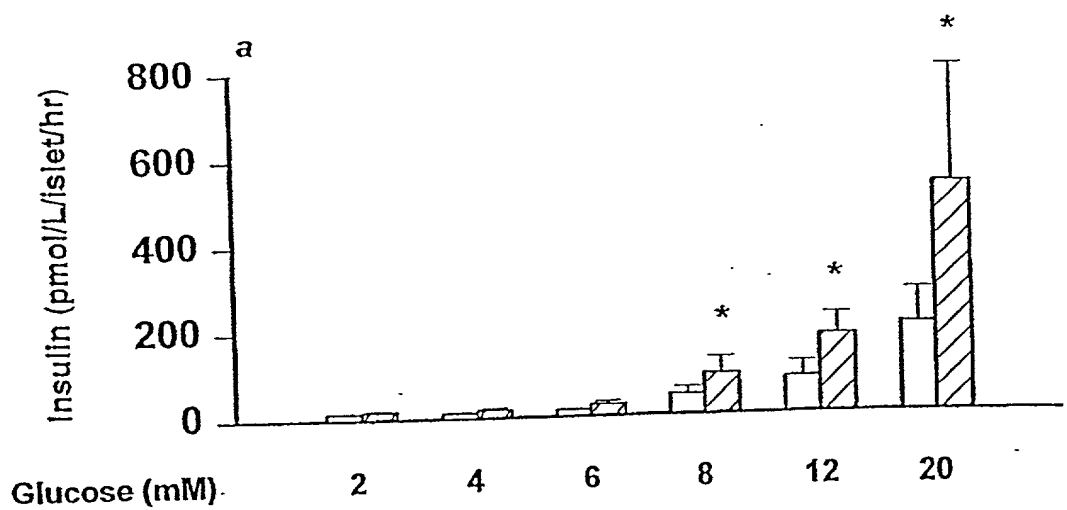


FIG. 9

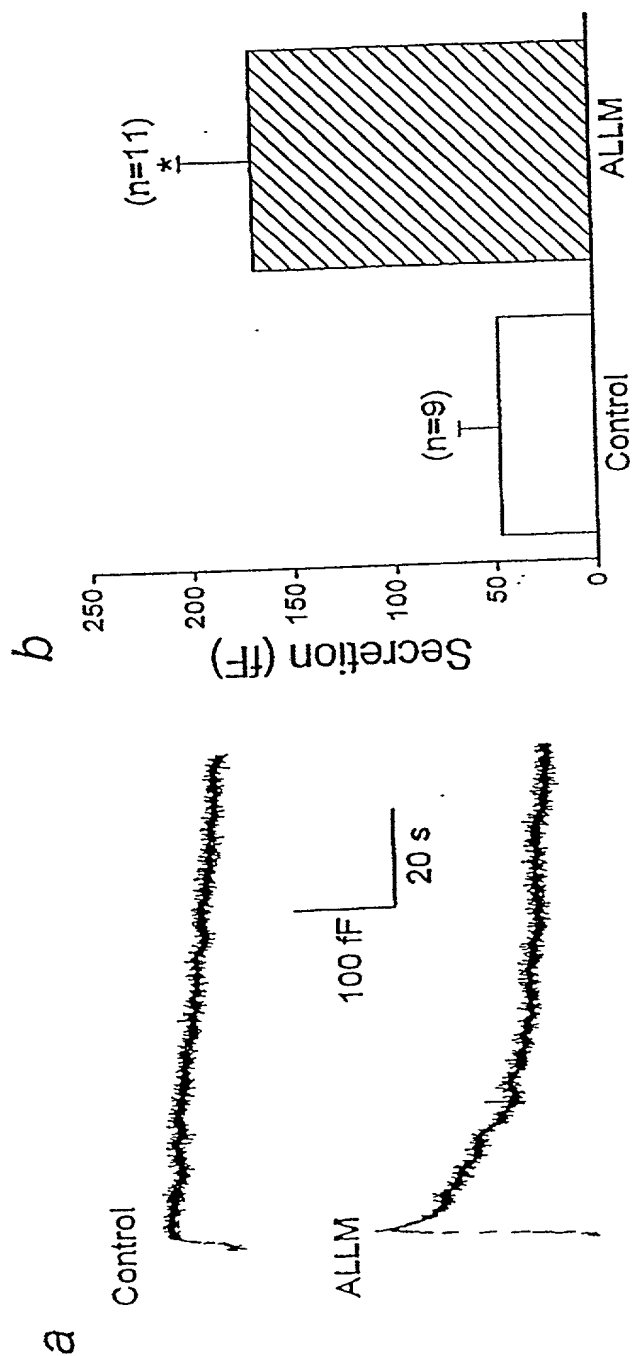


FIG. 10

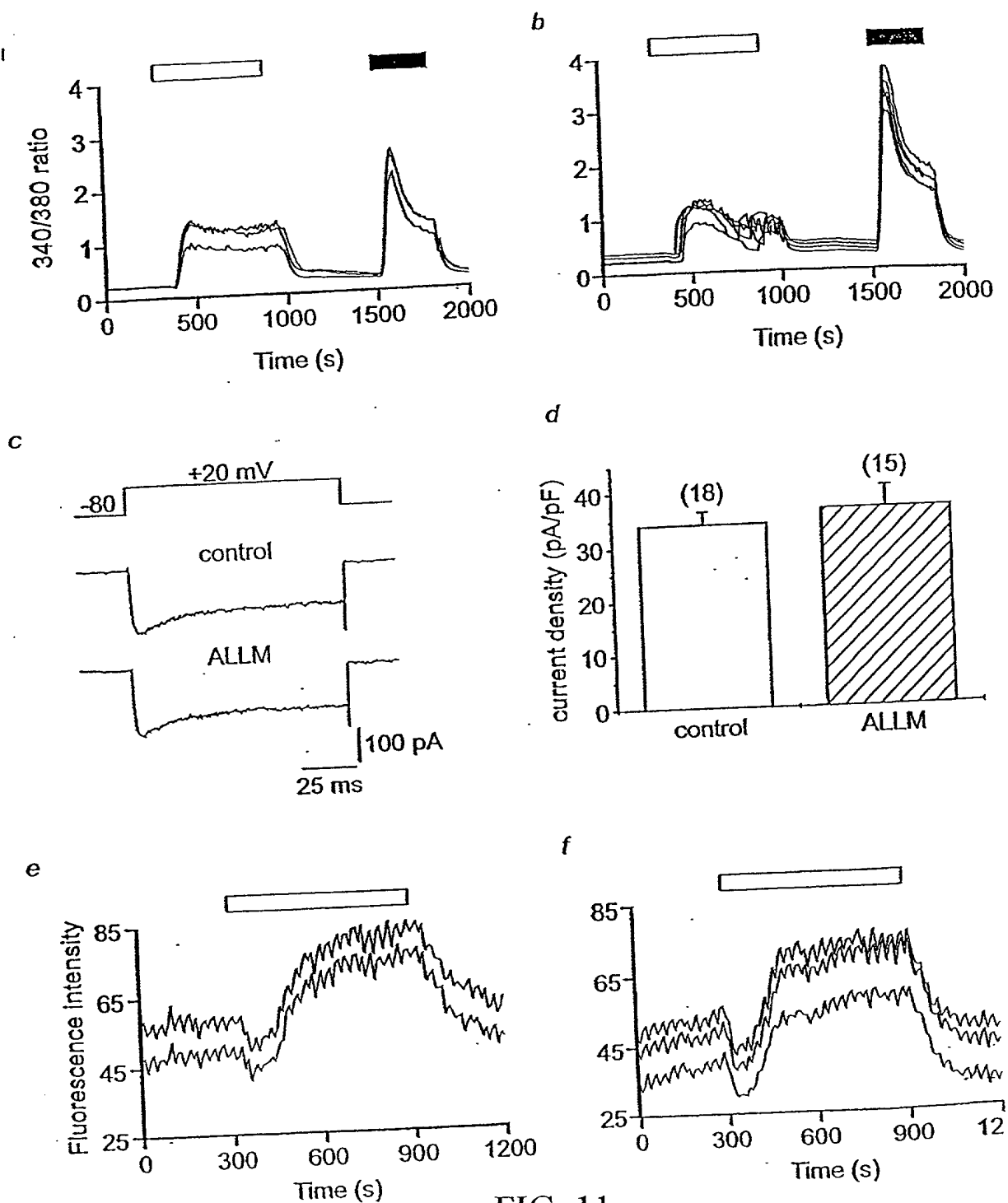


FIG. 11

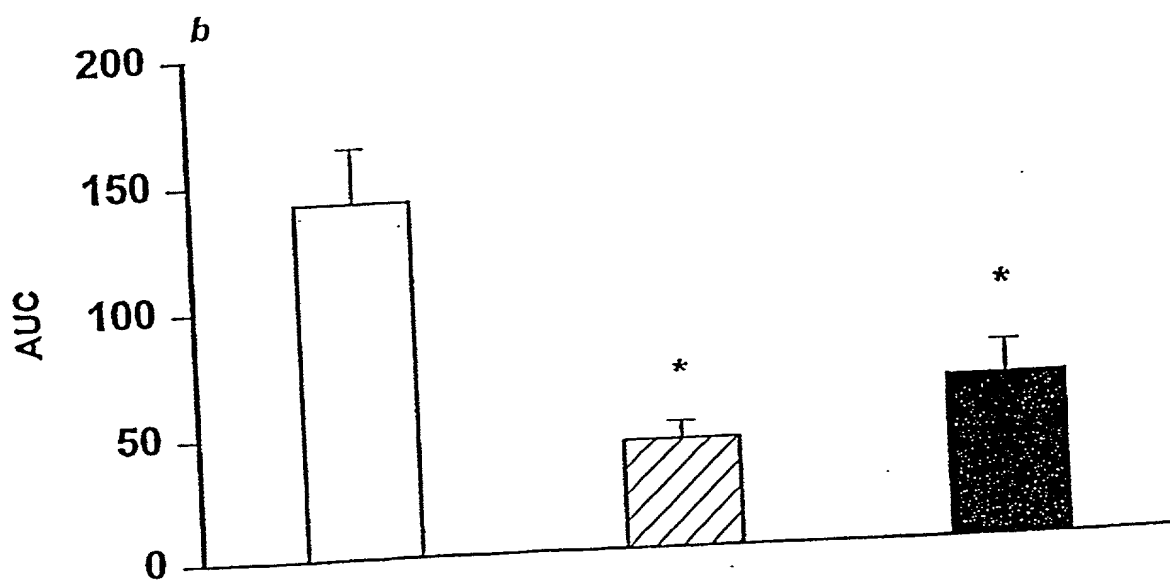
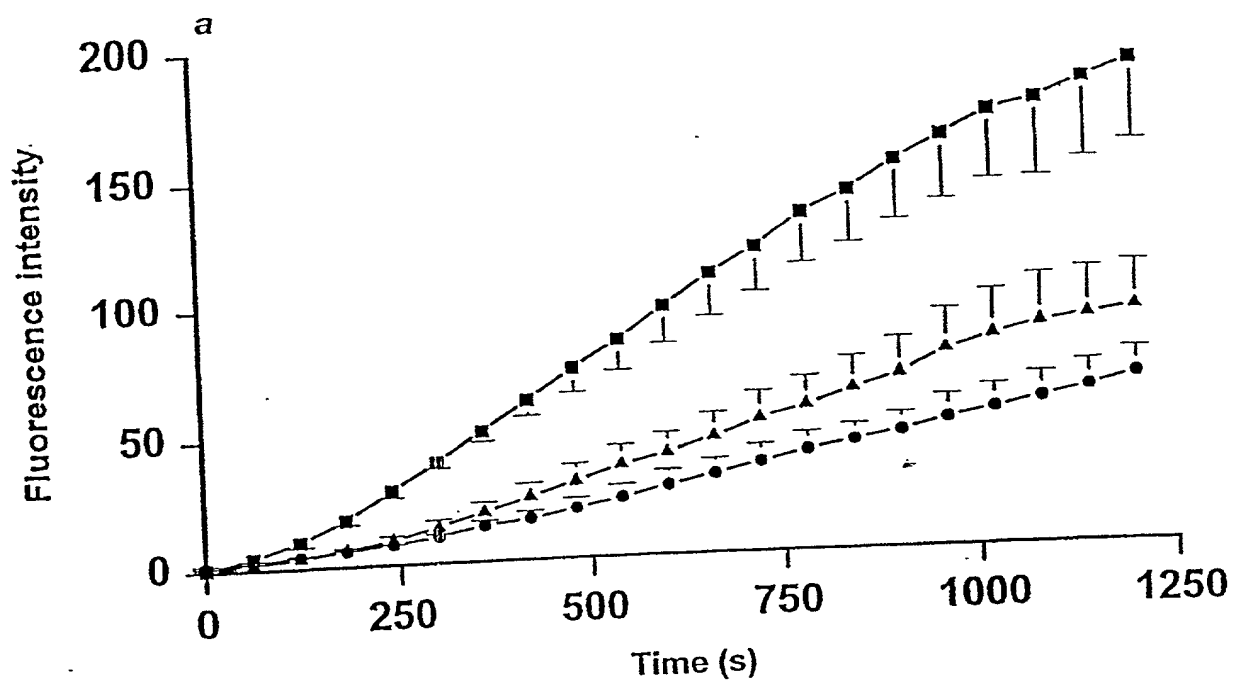


FIG. 12

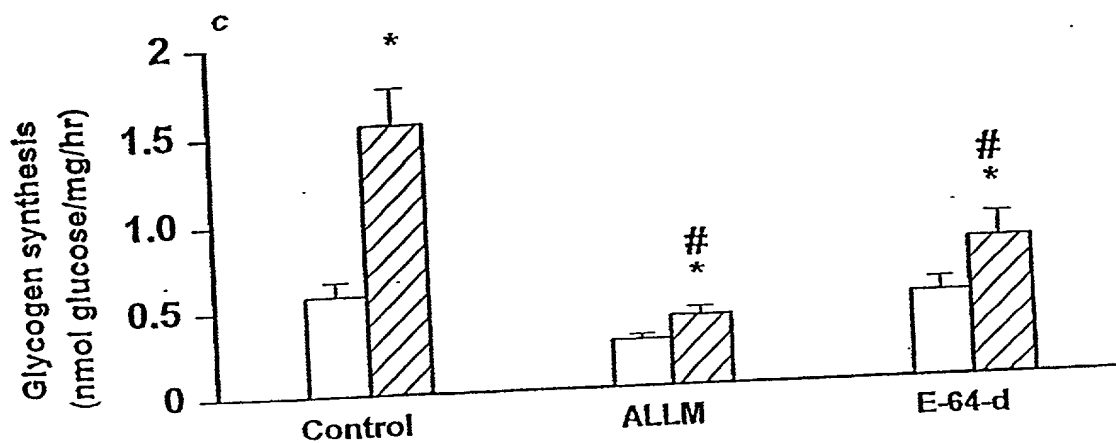
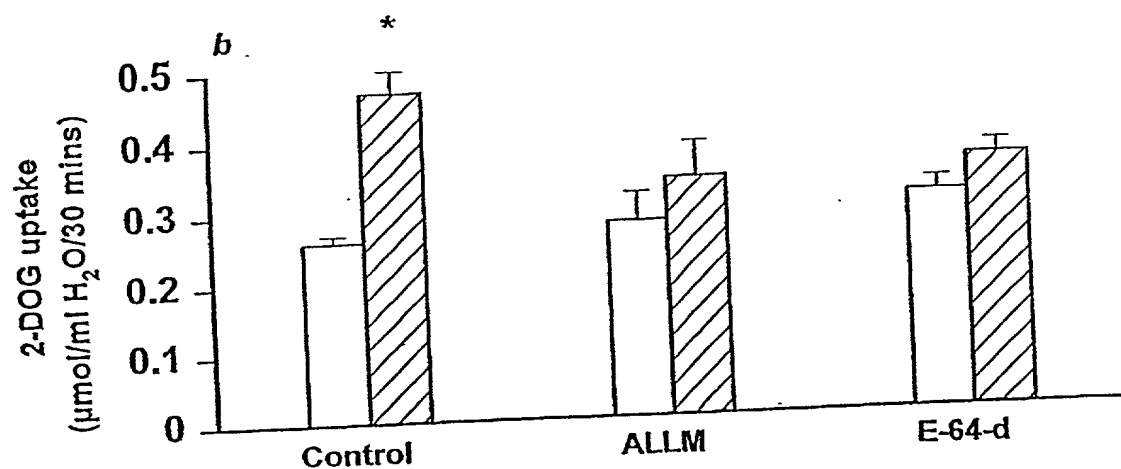
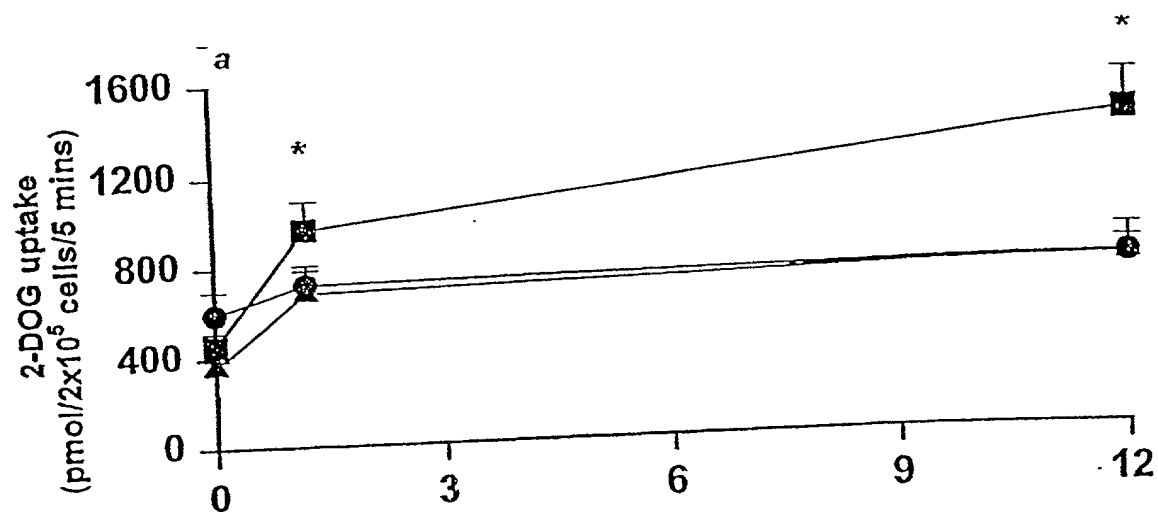
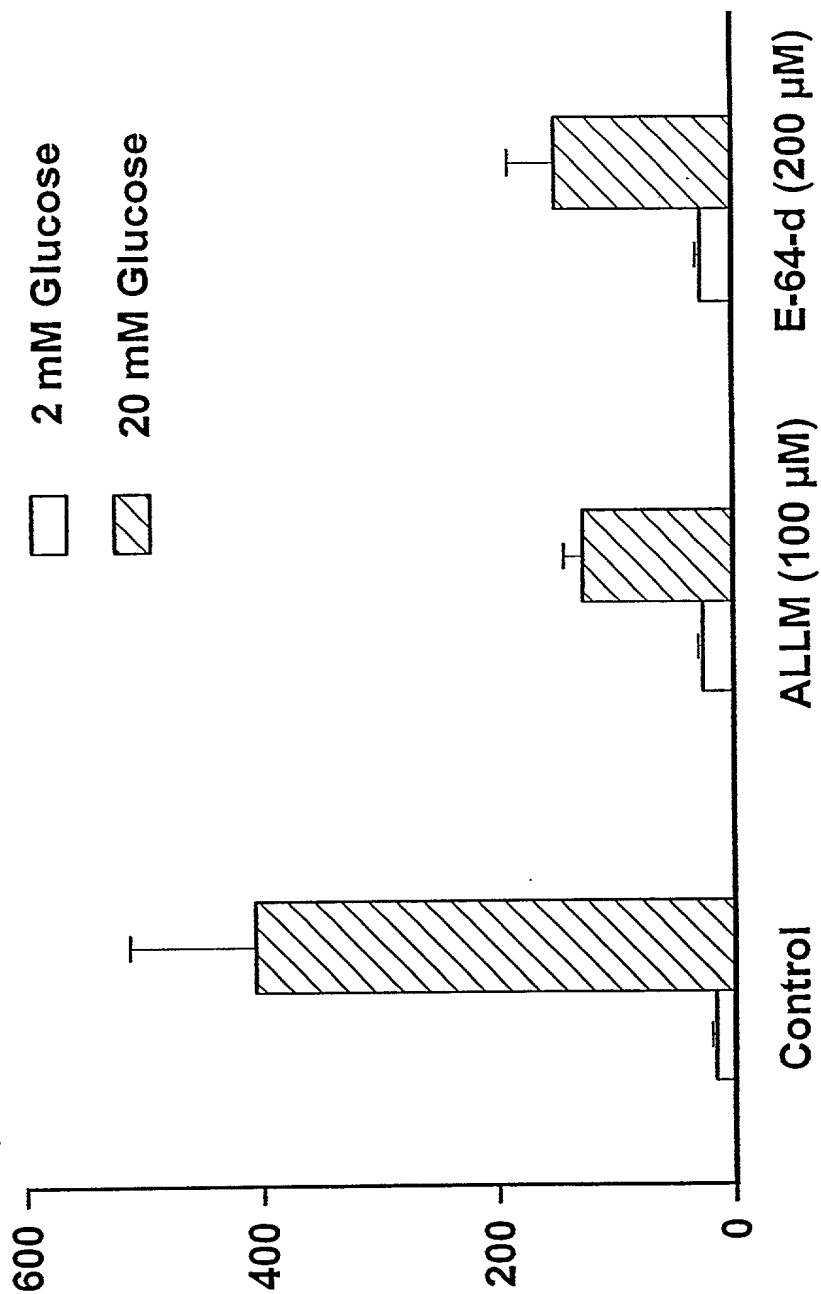
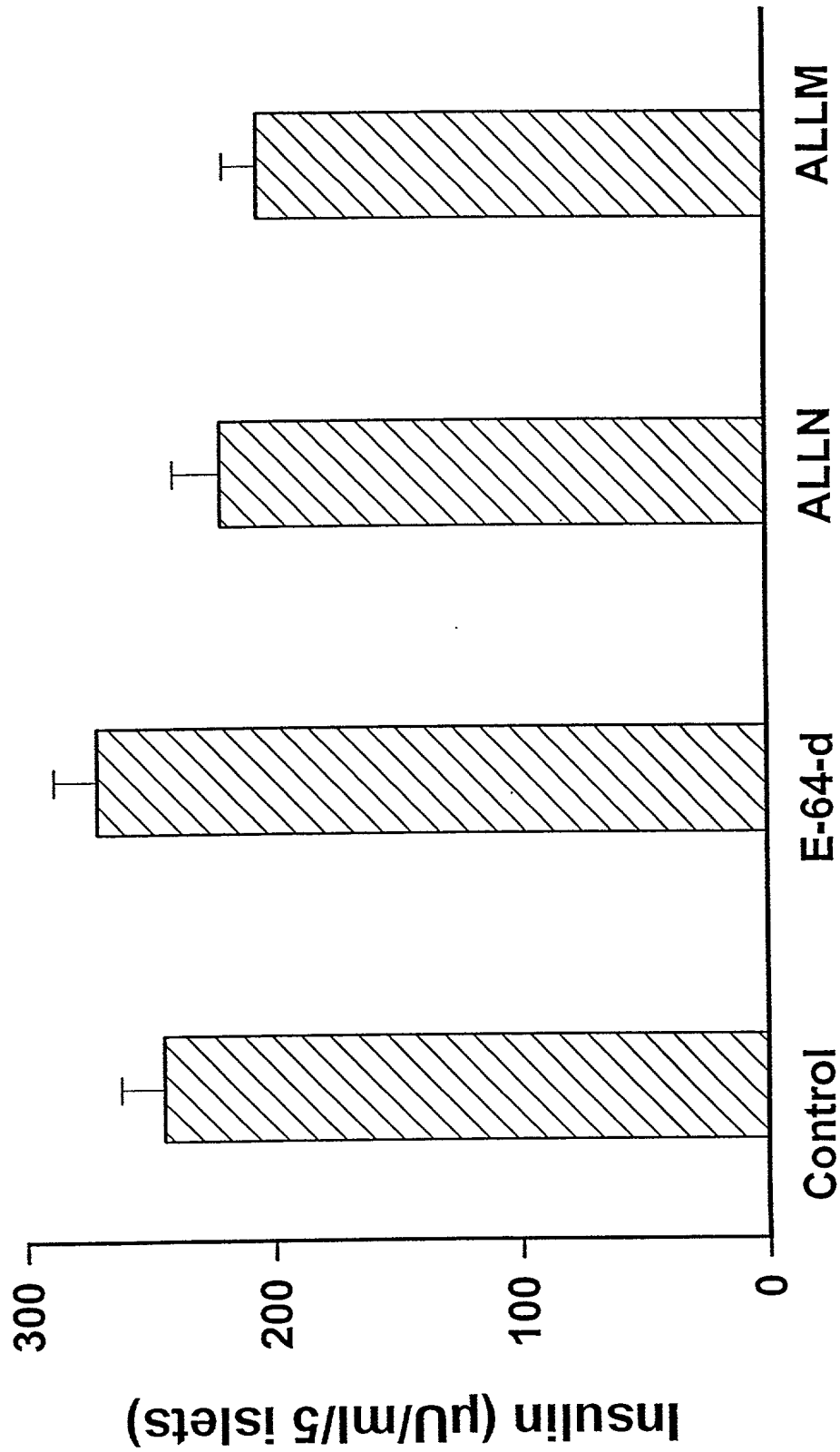


FIG. 13

Fig14 Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion



**Fig 15** Insulin content in 48 hour cultured islets (n=4)



**Fig16.** ALLM dose response in 48 hour treated islets

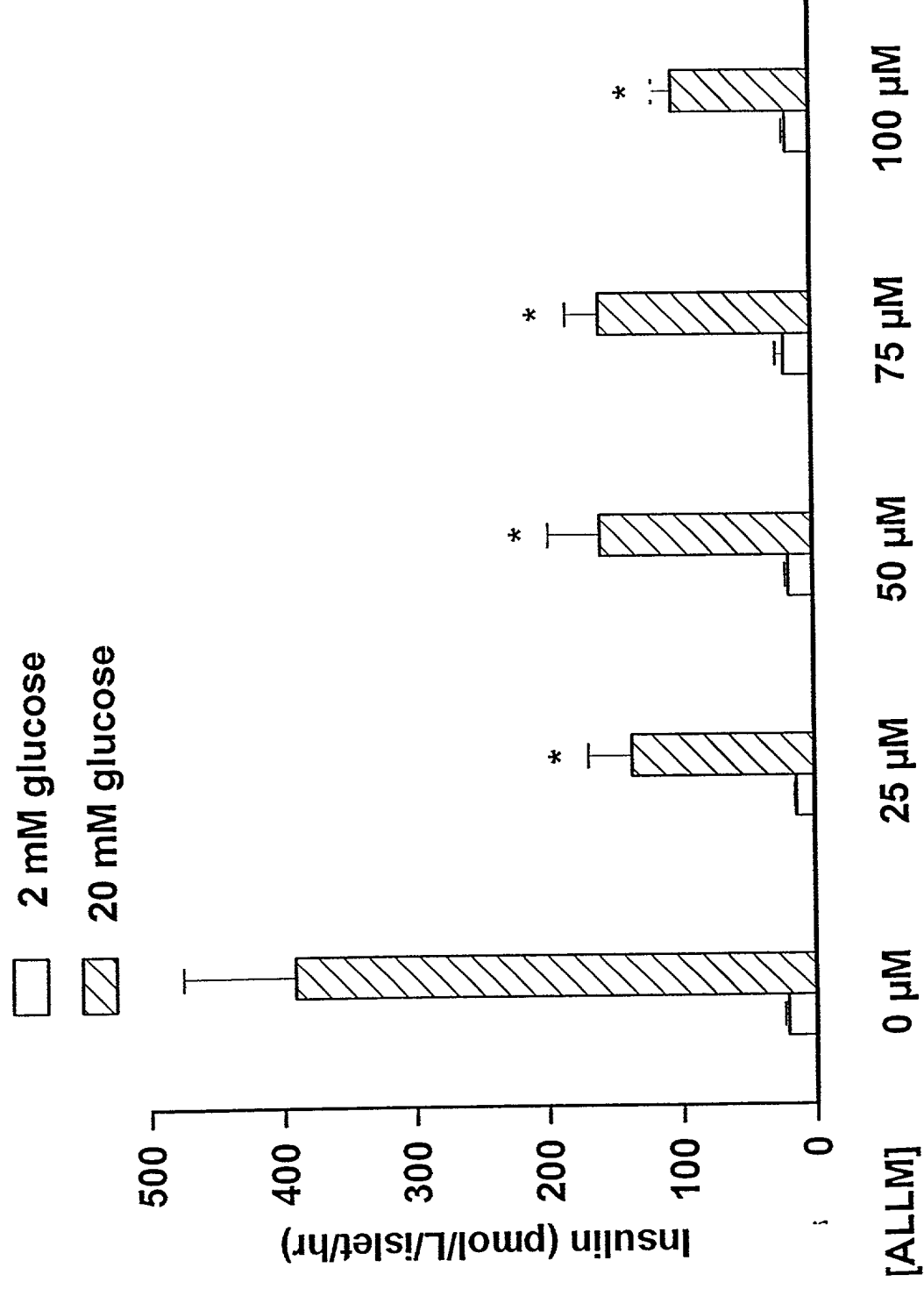
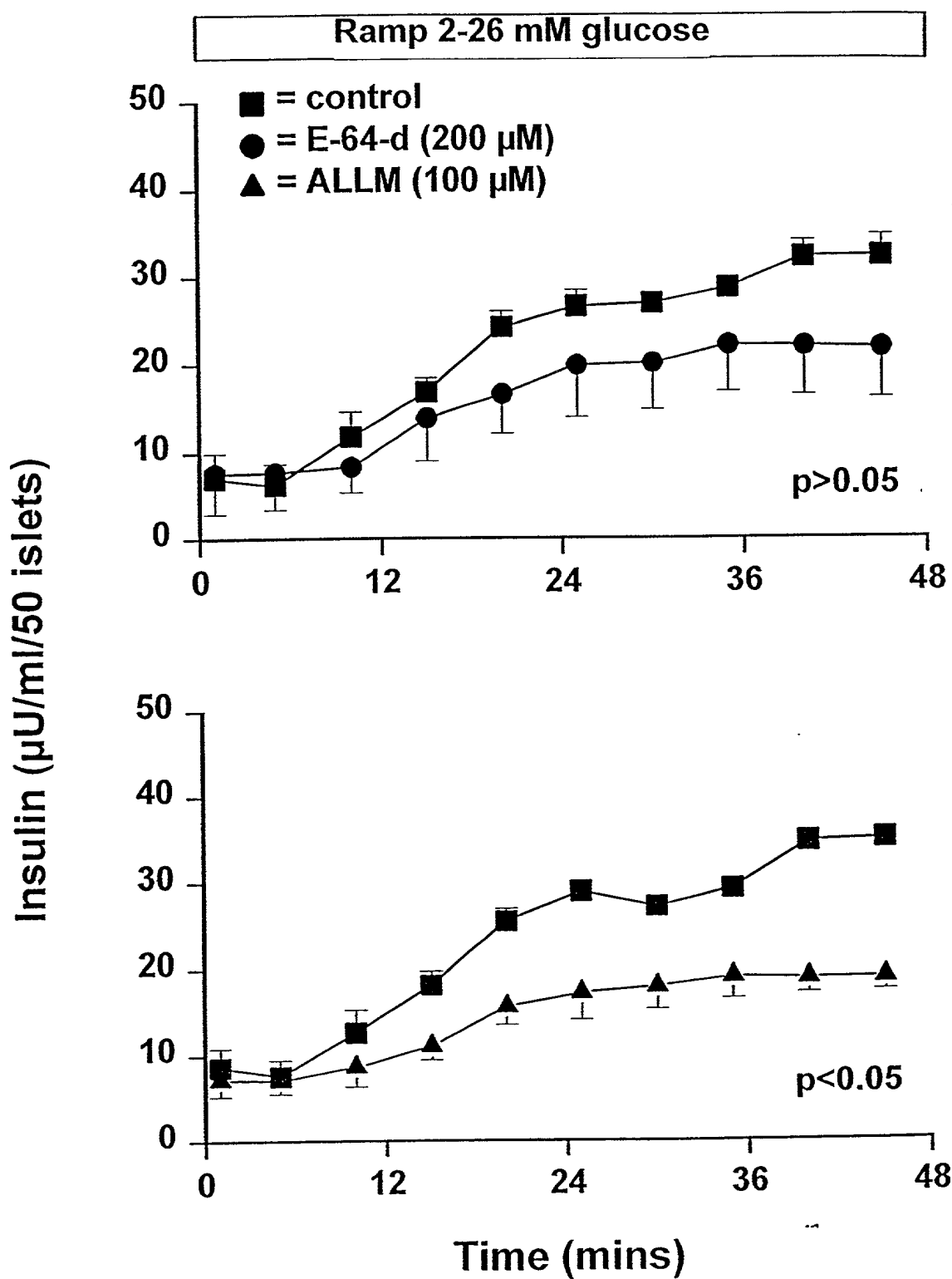




Fig17. Perifusion of 48 hour cultured islets (n=4)



# Insulin secretion in ALLM or E64-d treated mouse islets: Reversal study

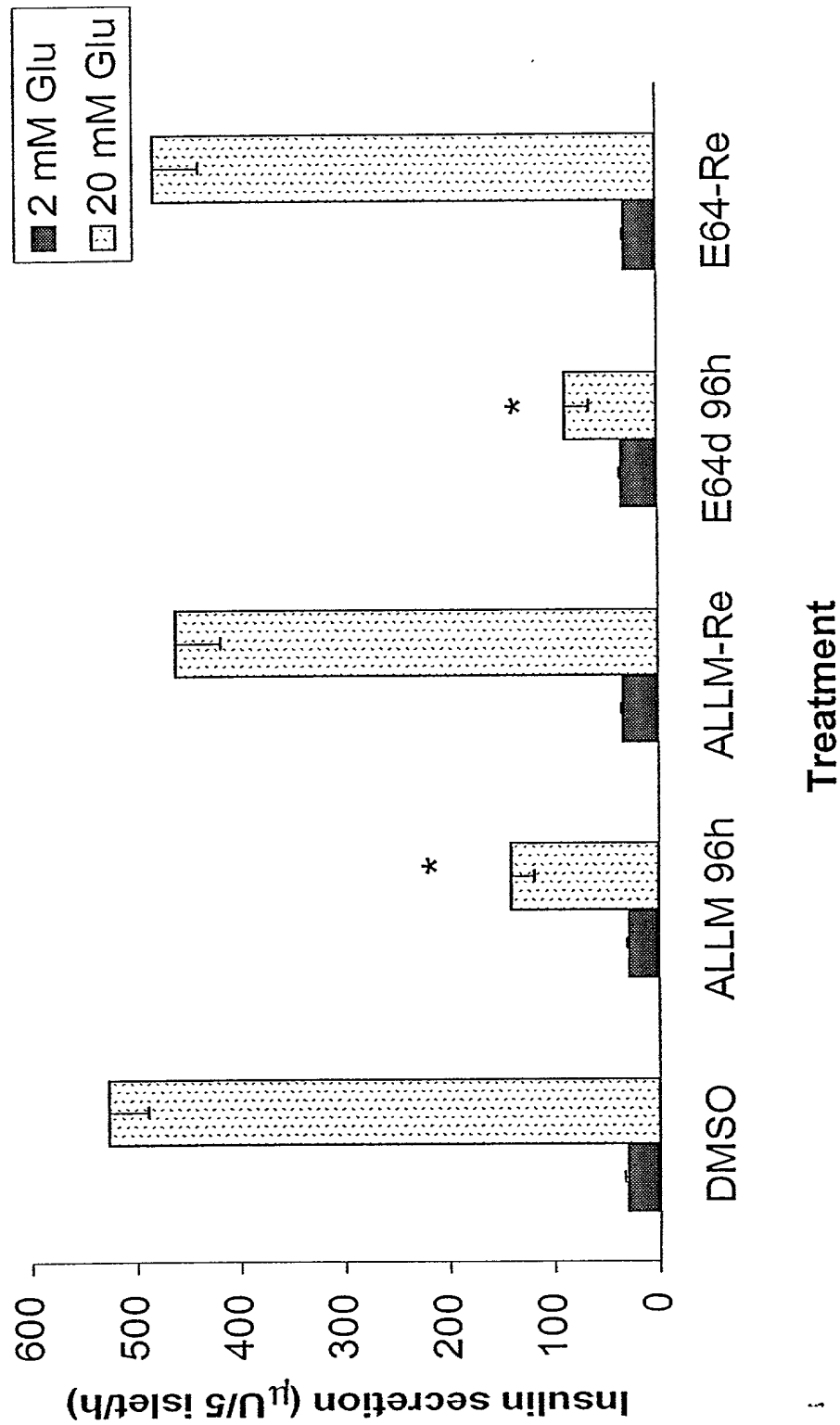
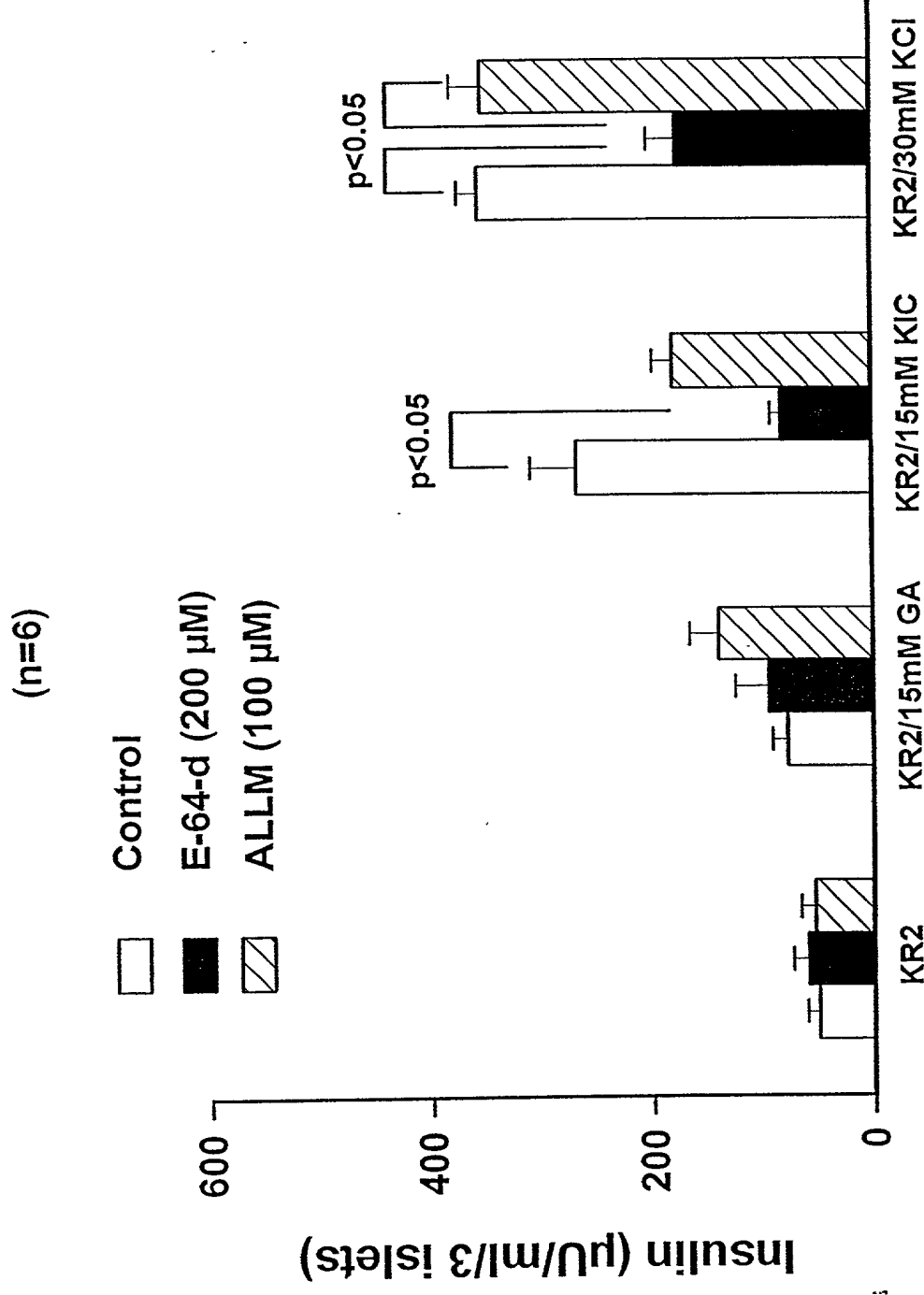


Fig. 18

Fig19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs



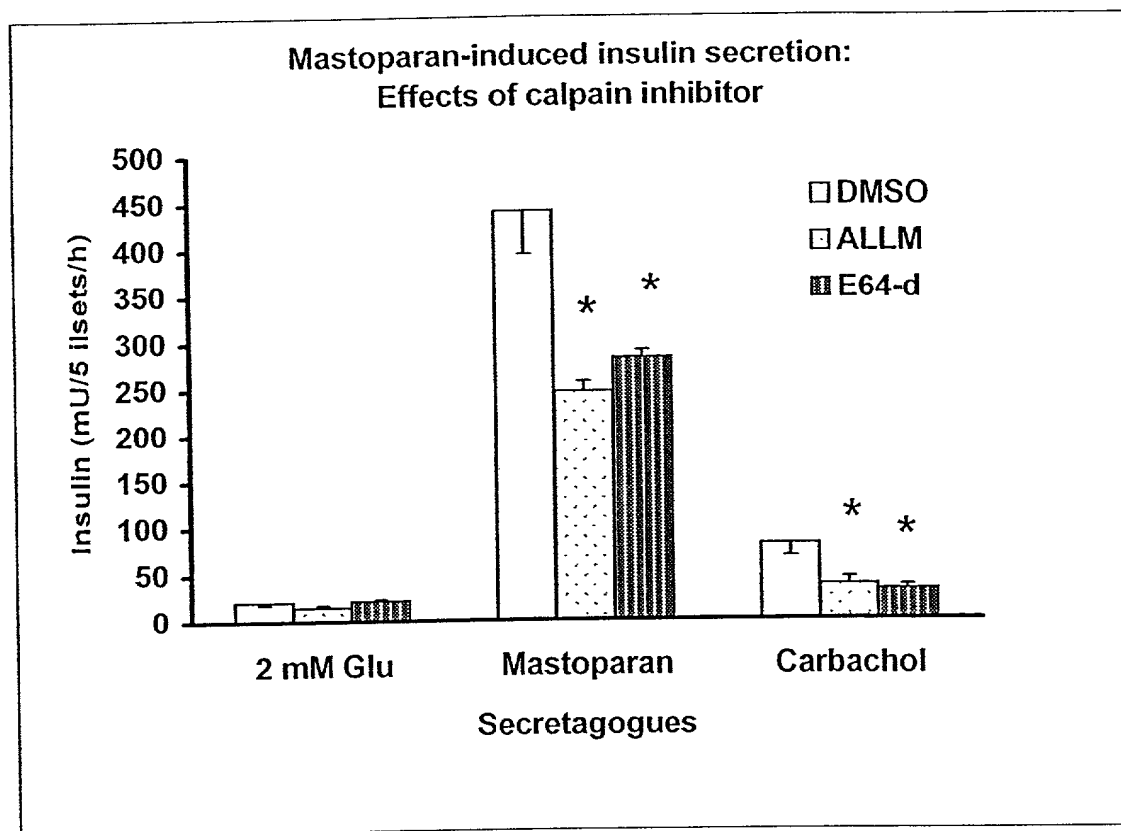
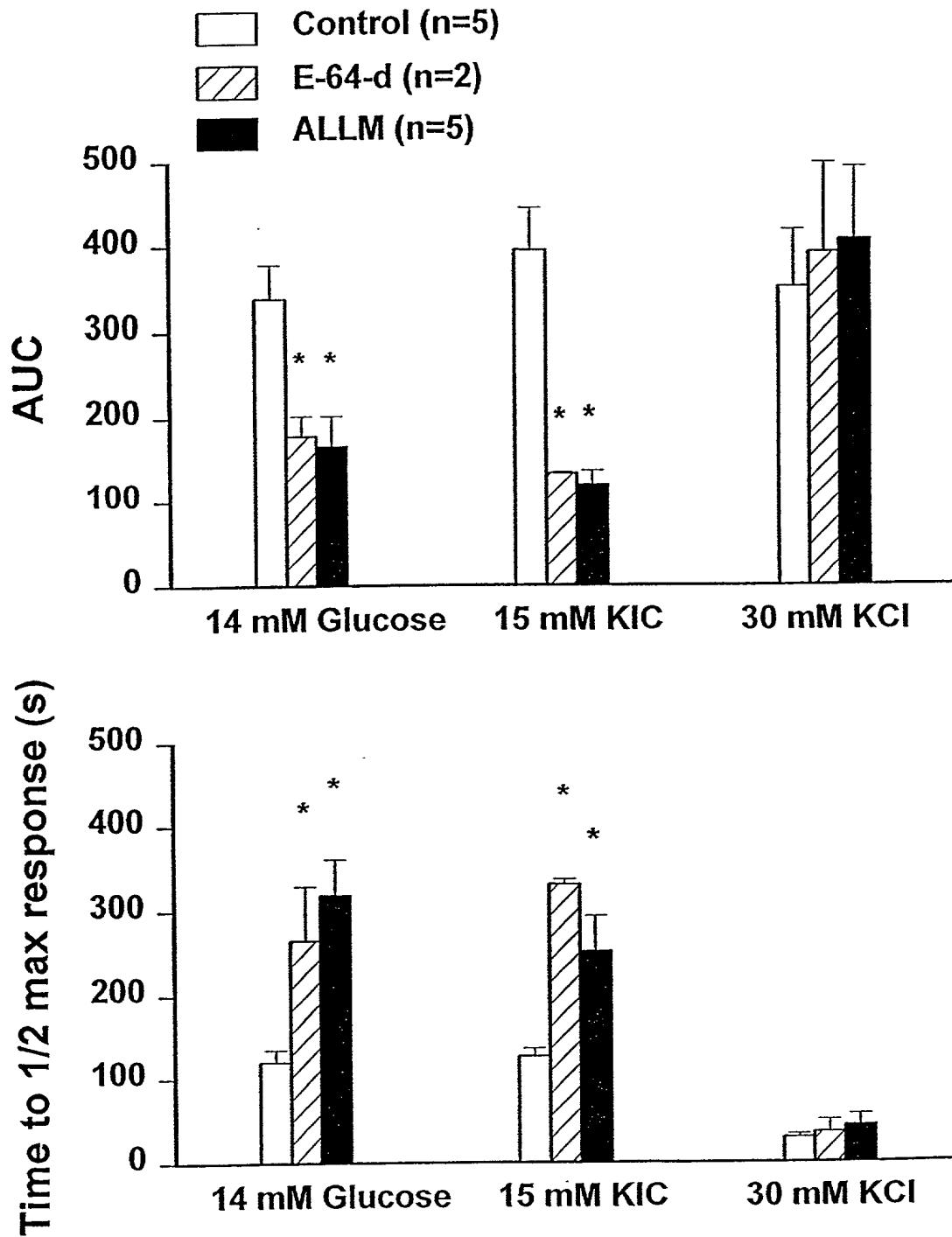


FIG. 20

Fig 21.  $[C^{2+}]_i$  responses to glucose, KIC and KCl



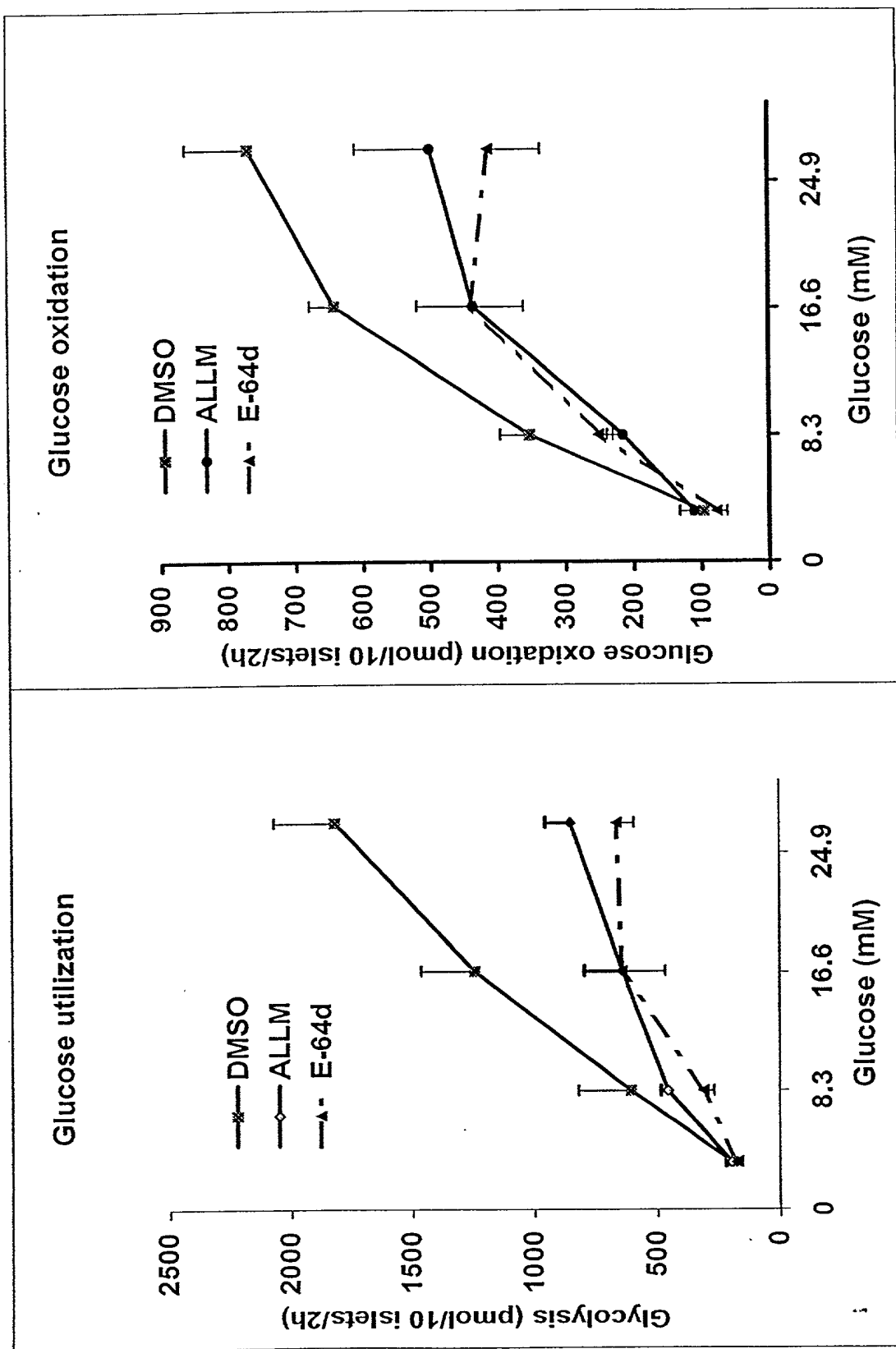
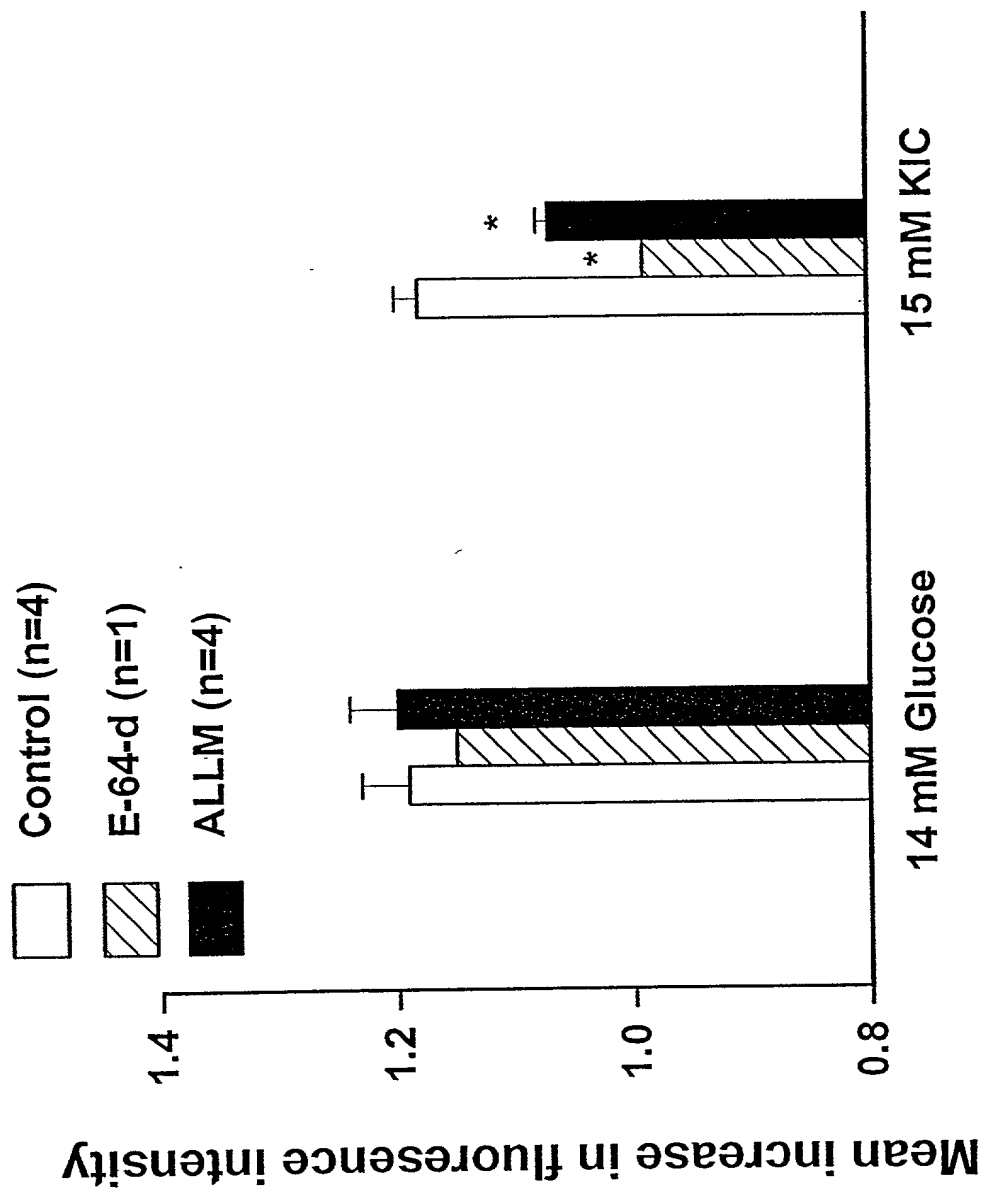


Fig. 22

**Fig 23. NAD(P)H responses to glucose and KIC**



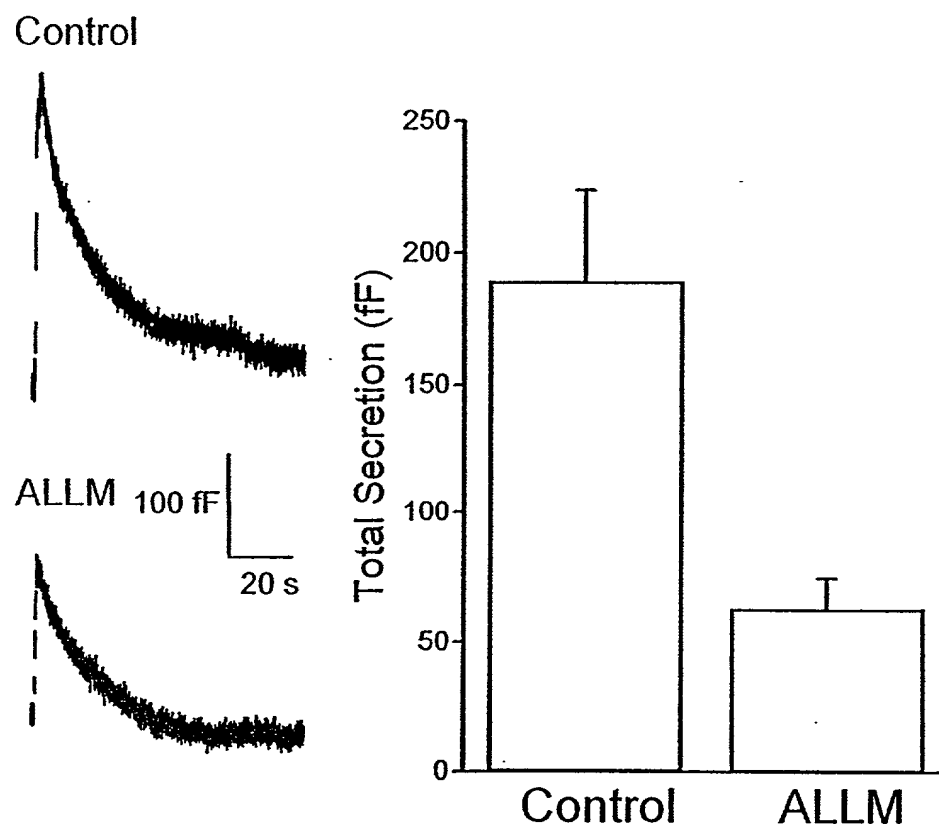


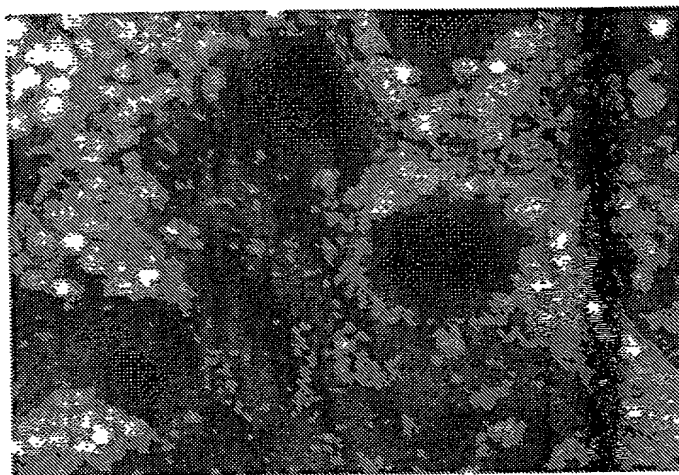
Fig. 24. Measurement of membrane capacitance in isolated  $\beta$ -cells



DMSO



E64d



ALLM

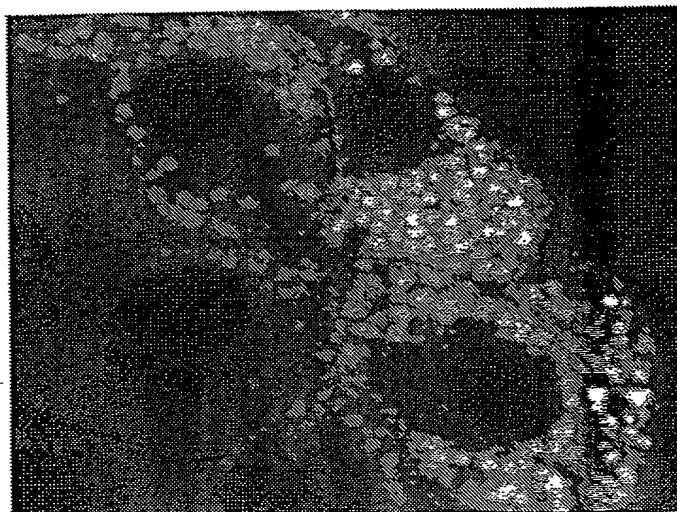


FIG. 25

09768877.08101

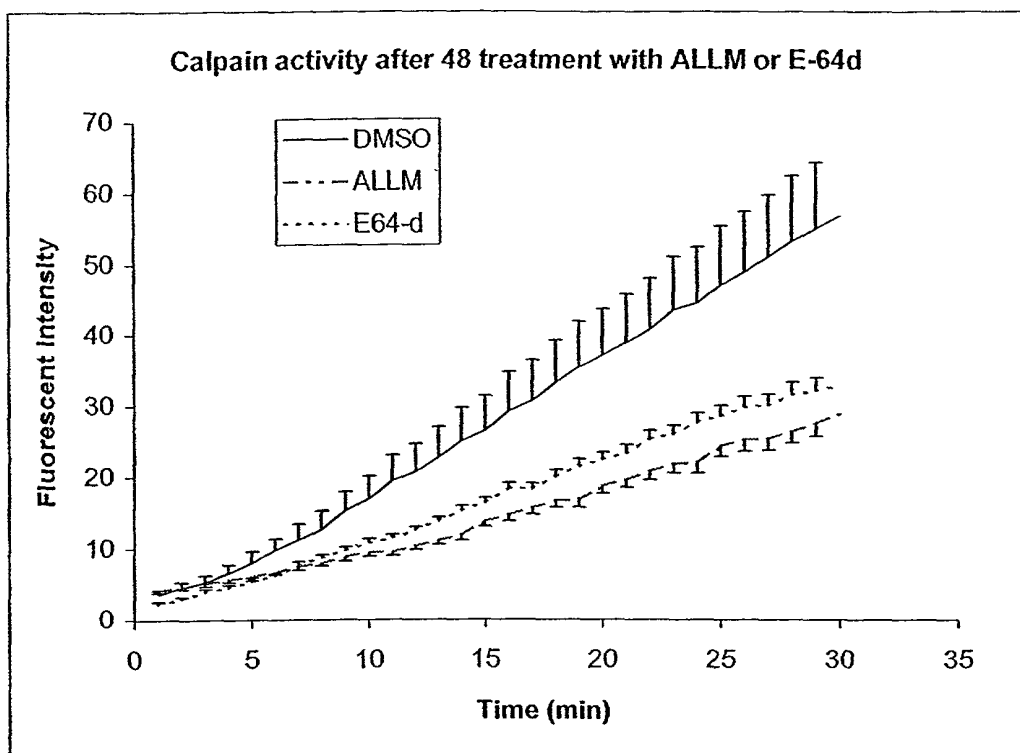


FIG. 26